REMARKS

Applicants respectfully request entry of the amendments and remarks submitted herein. Claims 7-9, 11-15, and 40-48 are currently pending. Attached is a marked-up version of the changes being made by the current amendments. Reconsideration of the pending application is respectfully requested.

Sequence Compliance

The Examiner indicated that the application does not comply with the requirements of 37 CFR §1.821-1.825. Applicants have amended the specification to include a sequence identifier for the consensus sequence shown on page 12, and herein provide a new paper copy of the Sequence Listing and a new computer readable form. A verified statement also is provided indicating that the contents of the paper copy and the computer readable form are identical. Applicants submit that the application is now in compliance with 37 CFR §1.821-1.825.

The Specification

The specification stands objected to for containing an embedded hyperlink. This objection is respectfully traversed.

Applicants have amended the specification to remove the references to embedded hyperlinks on pages 12 and 13.

The Examiner further indicated that the trademarks referred to on pages 23 and 25 need to be capitalized and need to be accompanied by the generic terminology. Applicants have amended the specification at pages 22-23 to capitalize the names of BlockAce™ and the EZ-Link™ Plus Activated Peroxidase kit, and to indicate the generic components of the latter. In addition, the specification at page 25 has been amended to capitalize the name of the CellTiter 96® kit, to indicate it's components, and to indicate that the ECL™ Western blotting reagents are trademarked.

Applicants note that BlockAce™ and the ECL™ Western blotting reagents are likely proprietary, as the literature associated with each reagent does not disclose the respective

components. The Examples in the instant application accurately describe the Western blot methods used by the inventors. In addition to using the ECL™ Western blotting reagents from Amersham, however, there are numerous other Western blot methods and reagents known to those of ordinary skill in the art. Further, the procedure of using a blocking agent during hybridizations or binding reactions is well known in the art, and many different blocking agents are available.

Applicants have amended the specification to comply as fully as possible with MPEP §608.01. In view of the amendments and the remarks herein, Applicants respectfully request that the objection to the specification be removed.

The 35 U.S.C. §112 Rejections

Claims 7-9, 11-15 and 40-48 stand rejected under 35 U.S.C. §112, first paragraph, as the Examiner asserted that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. Applicants respectfully traverse this rejection.

The Examiner stated that the specification, while being enabling for methods encompassed by the claims involving ECE-1a and ECE-1b, does not reasonably provide enablement for such methods involving any ECE polypeptide (page 4 of the Office Action). The Examiner also stated that the "instant specification does not provide meaningful information on how to use the claimed method employing other forms of ECE as Aβ degrading enzymes except for ECE-1a and ECE-1b, thus, requiring undue experimentation on part [*sic*] of one skilled in the art to discover how to practice the claimed invention" (page 4 of the Office Action).

Contrary to the Examiner's statements, Applicants' specification clearly enables the use of any ECE polypeptide in the methods of the invention. For example, Applicants respectfully refer the Examiner to page 7, lines 7-9 for a definition of ECE polypeptides. The functional definition of ECE polypeptides as disclosed on page 7 is further supported by the disclosure of methods to detect Aβ (please see page 14, line 1 through page 16, line 7) and methods of performing a big ET assay (please see page 6, lines 6-9). In addition, Applicants respectfully refer the Examiner to page 7, lines 11-12, which discloses ECE polypeptides that had been identified at the time the instant application was filed. Applicants refer the Examiner to page 7,

lines 12-27, which discloses the GenBank Accession Numbers of representative ECE polypeptides, and to page 8, lines 11-12, which discloses the GenBank Accession Numbers of representative nucleic acid sequences encoding ECE polypeptides. The specification at page 7, lines 27-31, also discloses how to obtain an ECE polypeptide sequence from an ECE nucleic acid sequence. Further, Applicants respectfully refer the Examiner to page 12, line 14 through page 13, line 9, which discloses the features and characteristics of typical ECE polypeptides. Such features and characteristics can be used to identify an ECE polypeptide.

The Examiner indicated in the Office Action that the state of the prior art is such that there is no indication that all of the proteins encompassed by the term "ECE" would be involved in the processing of the Aβ peptide (page 5 of the Office Action). The Examiner further indicated that "[b]ecause the instant specification fails to provide neither such guidance nor working examples of a method, which employs an ECE other than ECE-1a or ECE-1b, a skilled artisan would have to resort to undue experimentation to practice the full scope of the claimed invention" (page 5 of the Office Action).

The standard is not whether or not experimentation is required, but how much experimentation is required. "Enablement is not precluded by the necessity for some experimentation...However, experimentation needed to practice the invention must not be undue experimentation" (*In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988), citations omitted). The specification discloses assays for functionally identifying and/or evaluating the activity of ECE polypeptides using either hydrolysis of Aβ or conversion of ET precursors into mature ET (see, for example, page 6, and pages 14-16). In addition, the specification discloses features and characteristics of ECE polypeptides that can be used to identify an ECE polypeptide (see, for example, page 12, line 14 through page 13, line 9). Therefore, the specification provides a significant amount of guidance such that a skilled artisan could practice the claimed invention without undue experimentation.

The Examiner additionally indicated that since "substrate specificity is a major issue in proteolytic activity of enzymes, and some structurally related proteases can exhibit different activity toward the same, for example, biologically active peptides, like ECE-1 and neprilysin, one of ordinary skill in the art would readily recognize the unpredictability of using different isoforms of ECE for possible cleavage of Aβ" (pages 5-6 of the Office Action).

The ECE-1a, -1b, -1c, and -1d isoforms are produced from a single gene using alternate promoters and, therefore, possess the same catalytic domain (see, for example, Valdenaire et al., 1999, *Eur. J. Biochem.*, 264:341-9 (copy enclosed) and references therein). Further, ECE-1 and ECE-2 are highly homologous and share similar catalytic activity (see Emoto & Yanagisawa, 1995, *J. Biol. Chem.*, 270:15262-8 (copy enclosed)). In addition, Applicants examined the cleavage of Aβ by ECE using a soluble ECE-1 (see, for example, Example 11 of the instant specification and Figure 7 of Eckman et al., 2001, *J. Biol. Chem.*, 276:24540-8 (copy enclosed)). These experiments revealed that the soluble ECE-1 exhibits preferential cleavage of Aβ at the amino side of hydrophobic residues, which is consistent with the known substrate specificity of ECE-1. Therefore, Applicants submit that cleavage of Aβ by a number of different ECE polypeptides is highly predictable. In addition, since the specification discloses methods for identifying and/or evaluating the Aβ hydrolysis capabilities of ECE polypeptides (please see page 14, line 1 through page 16, line 7, and Example 11), the art of determining whether or not an ECE polypeptide is capable of cleaving Aβ is highly predictable as well.

The Examiner further stated that there is a "total absence of working examples" (page 6 of the Office Action). Applicants submit that the Examiner is incorrect, and working examples are, in fact, provided in the instant application. Examples 1-12 describe experiments using ECE-1a, ECE-1b, a soluble ECE-1, and ECE-2. Contrary to the Examiner's statements, Applicants have in fact exemplified the claimed method using a representative number of ECE polypeptides, and have disclosed methods of determining whether or not an ECE polypeptide is suitable for use in the claimed methods. Therefore, Applicants have provided both direction and guidance in identifying suitable ECE polypeptides and using such polypeptides in the claimed methods.

Based on the above remarks, identifying an ECE polypeptide and determining if such a polypeptide cleaves either or both Aβ or big-ET would not require undue experimentation by one of ordinary skill in the art. The specification discloses how to practice the claimed invention using any polypeptide that has been identified as an ECE polypeptide. Applicants submit that the specification is clearly enabled for using any ECE polypeptide in a method of the invention. Therefore, Applicants respectfully request that the rejection of claims 7-9, 11-15 and 40-48 under 35 U.S.C. §112 be withdrawn.

Claims 7-9 and 40-41 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Applicants have herein amended claim 7 to clarify that the acronym "ECE" refers to "endothelin converting enzyme." Applicants submit that claim 7 is not indefinite. Therefore, Applicants respectfully request that the rejection of claims 7-9 and 40-41 under 35 U.S.C. §112 be withdrawn.

CONCLUSION

Applicants ask that claims 7-9, 11-15, and 40-48 be allowed. Enclosed is a $55 check for a One-Month Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: November 15, 2002

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The specification on page 12, lines 10-27 has been amended as follows:

Site-directed mutagenesis previously has been performed on ECEs, as well as on related zinc metalloendopeptidases (*e.g.*, neprilysin (NEP), thermolysin) to identify important residues (see Shimada et al., 1996, *Biochem J.*, 315:863-7; Hoang et al., 1997, *Biochem J.*, 327:925-9; Savage et al., 1998, *J. Cardiovasc. Pharmacol.*, 31(Suppl.):S16-18; Marie-Claire et al., 1999, *J. Mol. Biol.*, 285:1911-5). A typical ECE-1, for example, has a short N-terminal tail (about 50 residues), one or more hydrophobic transmembrane domains (usually about 20 residues) and a large domain that includes a zinc-binding motif common to catalytic domains of metalloproteases (about 680 residues). The residues acting as zinc ligands and those involved in the catalytic activity are known, and include two histidines (H) that bind zinc and are very close together in the sequence, and an active site glutamic acid (E) residue C-terminal to the first histidine that acts as a nucleophile and promotes the attack on the carbonyl carbon of the substrate by a water molecule. The consensus pattern for ECE enzymes is as follows: [GSTALIVN]-x(2)-H-E-[LIVMFYW]-{DEHRKP}-H-x-[LIVMFYWGSPQ] (SEQ ID NO: 8) (ProSite Accession No. PS00142 and ProSite Documentation PDOC00129; for information regarding ProSite prefixes, refer to Sonhammer et al. (1997) *Protein* 28:405-420[or http://www.expasy.ch]). ECE-1 also has a number of cysteine residues that are conserved among, at least one of which is involved in disulphide-linked homodimerization and other(s) that can be palmitoylated.

The paragraph bridging pages 12 and 13 has been amended as follows:

A representative human ECE-1 amino acid sequence (*i.e.*, NM 001397) is predicted to have the following: ten putative N-glycosylation sites (PS00001); one putative glycosaminoglycan attachment site (PS00002); three putative cAMP- and cGMP-dependent protein kinase phosyphorylation sites (PS00004); ten putative protein kinase C phosphorylation sites (PS00005); sixteen to eighteen putative casein kinase II phosphorylation sites (PS00006); three putative tyrosine kinase phosphorylation sites (PS00007); nine to ten putative N-

myristoylation sites (PS00008); and one putative neutral zinc metallopeptidase zinc-binding region signature domain (PS00142)[(see www.expasy.ch for the sequence of putative consensus sites and their respective positions in a given ECE amino acid sequence)]. In addition, glycosylation can be important for full enzymatic activity of ECE-1. The above examples are representative only, and those of skill are aware that any position along an ECE nucleic acid sequence represents a site of potential mutation.

The specification at the paragraph bridging pages 22 and 23 has been amended as follows:

In particular, the sandwich ELISA used herein was performed as follows: 96-well microtiter plates were coated overnight at 4°C with 100 µl of a 5 µg/ml dilution of primary antibody in sodium carbonate coating buffer (SCCB; 0.1 M Na_2CO_3, pH 9.6). Plates were blocked overnight at 4°C with 300 µl of [Block Ace] BLOCKACE Solution (PBS + 1.0% [Block Ace] BLOCKACE (Snow Brand Milk Products, Japan), 0.05% NaN_3, pH 7.4). Samples for analysis and synthetic Aβ standards (Bachem, Switzerland) were diluted in buffer EC (0.02 M NaH_2PO_4, 0.002 M EDTA, 0.4 M NaCl, 0.2% BSA, 0.05% CHAPS, 0.04% [Block Ace] BLOCKACE, 0.05% NaN_3, pH 7.0) and allowed to incubate on the plates overnight at 4°C. Plates were washed twice with PBS (8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 139 mM NaCl, 2.7 mM KCl, pH 7.4) and 100 µl of secondary antibody directly coupled to HRP ([Pierce EZ-link] EZ-LINK™ Plus Activated Peroxidase kit (contents of kit: peroxidase, 5 M sodium cyanoborohydride solution ($NaBH_3CN$), quenching buffer (3 M ethanolamine, pH 9.0), BupH™ phosphate buffered saline, Buph™ carbonate-bicarbonate buffer), according to manufacturers directions; Pierce Chemical Co., Rockford, IL) was allowed to bind either 4 hrs at room temperature or overnight at 4°C. Plates were then washed twice with PBS containing 0.05% Tween 20 followed by two additional washes in PBS. Detection was performed using TMB (3,3',5,5'-tetramethyl-benzidine) as an HRP substrate according to the manufacturer's specifications (Kirkegaard & Perry Laboratories (KPL), Gaithersburg, MD) and the reaction stopped by the addition of 100 µl of 1N H_3PO_4. Plates were read at 450 nm in a SpectraMax Plus spectrophotometer (Molecular Devices; Sunnyvale, CA) and analyzed by SOFTmax® PRO

software. Aβ40 or Aβ42 were quantitated by comparison with the values obtained for each synthetic Aβ standard from the same plate.

The specification on page 25, lines 1-16 has been amended as follows:

Cells were passaged into 6-well plates one day prior to treatment and grown to confluence. Triplicate wells were washed twice with Hank's balanced salt solution and then incubated for 17-24 hrs with 1 ml of growth medium containing phosphoramidon (34-100 μM), thiorphan (Sigma) or captopril (Sigma) at the appropriate concentrations. Control cells were incubated in growth medium containing an equal volume of vehicle (PBS). After treatment, the culture medium was harvested, spun at 14,000 xg, and the supernatant analyzed for Aβ40 or Aβ42 using sandwich ELISA and for secreted APP using Western blot. To assess cellular toxicity of the compounds, MTS assays ([CellTiter] CELLTITER 96® (contents: tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS[a]] and phenazine ethosulfate (PES)), Promega, Madison, WI), which measure the conversion of MTS to formazan by metabolically active cells, were performed on the cells after the indicated times. Culture medium was subjected to electrophoresis on 10-20% Tricine gels (Novex, Carlsbad, CA) and was subsequently transferred to Immobilon P (Millipore, Bedford, MA). Western blots on CHO cells were performed using a 22C11 antibody (Boehringer) to detect secreted APP. Bound antibody was detected by incubation with the appropriate HRP-linked secondary antibody (Amersham, Uppsala, Sweden) using ECL™ Western blotting reagents (Amersham) followed by exposure to X-ray film.

The Sequence Listing submitted on November 26, 2001 was replaced with the enclosed copy of the Sequence Listing.

In the Claims:

Claim 7 has been amended as follows:

7. (Twice Amended) A method of identifying a compound that increases the activity of an endothelin converting enzyme (ECE) [ECE] polypeptide, the method comprising:

contacting Aβ with an ECE polypeptide in the presence of said compound; and

detecting the amount of unhydrolyzed Aβ,

wherein a decrease in the amount of unhydrolyzed Aβ produced in the presence of said compound compared to the amount of unhydrolyzed Aβ produced in the absence of said compound is an indication that said compound increases the activity of an ECE polypeptide.